

U.S. Serial No.: 10/549,871
Examiner: Mohammad Y. Meah
GAU: 1652
Page 2 of 14

RECEIVED
CENTRAL FAX CENTER
AUG 13 2007

IN THE CLAIMS:

1. (Currently Amended) A method for producing ergosta-5,7-dienol, mevalonate, farnesyl pyrophosphate, geraniol phyrophosphate, squalene epoxide, 4-dimethylcholesta-8,14,24-trienol, 4,4-dimethylzymosterol, squalene, farnesol, geraniol, lanosterol, zymosterone and zymosterol, campesterol, pregnenolone, 17-OH pregnenoleone, progesterone, 17-OH-progesterone, 11-deoxycortisol, hydrocortisone, deoxycorticosterone and/or corticosterone and/or its biosynthetic intermediates and/or metabolites comprising culturing organisms which have

a reduced $\Delta 22$ -desaturase activity and
an increased HMG-CoA-reductase activity and
an increased activity of at least one of the activities selected from the group consisting of lanosterol C14-demethylase activity, squalene epoxidase activity and squalene synthetase activity

in comparison with the wild type organism.

2. (Currently Amended) The method as claimed in claim 1, wherein, in order to reduce the $\Delta 22$ -desaturase activity, the gene expression of a nucleic acid encoding a $\Delta 22$ -desaturase is reduced in comparison with the wild type organism.

3. (Previously presented) The method as claimed in claim 2, wherein an organism without a functional $\Delta 22$ -desaturase gene is used.

4. (Currently Amended) The method as claimed in claim 1, wherein, in order to increase the HMG-CoA reductase activity, the gene expression of a nucleic acid encoding an HMG-CoA reductase is increased in comparison with the wild type organism.

5. (Currently Amended) The method as claimed in claim 4, wherein, in order to increase gene expression, a nucleic acid construct comprising a nucleic acid encoding an HMG-CoA

U.S. Serial No.: 10/549,871
Examiner: Mohammad Y. Meah
GAU: 1652
Page 3 of 14

reductase is introduced into the organism and whose expression in the organism is subject to reduced regulation in comparison with the wild type organism.

6. (Previously presented) The method as claimed in claim 5, wherein the nucleic acid construct comprises a promoter which, in the organism, is subject to reduced regulation in comparison with the wild-type promoter.
7. (Previously presented) The method as claimed in claim 6, wherein the nucleic acid encoding an HMG-CoA reductase is a nucleic acid whose expression in the organism is subject to reduced regulation in comparison with the homologous, orthologous nucleic acid.
8. (Previously presented) The method as claimed in claim 7, wherein the nucleic acid encoding an HMG-CoA reductase is a nucleic acid which encodes the catalytic region of HMG-CoA reductase.
9. (Currently Amended) The method as claimed in claim 8, wherein the nucleic acids introduced are nucleic acids encoding proteins comprising the amino acid sequence SEQ. ID. NO. 4 ~~or a sequence derived from this sequence by substitution, insertion or deletion of amino acids which has at least 30% identity with the sequence SEQ. ID. NO. 4 at the amino acid level, which proteins have the enzymatic characteristic of an HMG CoA reductase.~~
10. (Previously presented) The method as claimed in claim 9, wherein a nucleic acid comprising the sequence SEQ. ID. NO. 3 is introduced.
11. (Withdrawn) The method as claimed in any claim 1, wherein, in order to increase the lanosterol C 14-demethylase activity, the gene expression of a nucleic acid encoding a lanosterol C14-demethylase is increased in comparison with the wild type.

U.S. Serial No.: 10/549,871
Examiner: Mohammad Y. Meah
GAU: 1652
Page 4 of 14

12. (Withdrawn) The method as claimed in claim 11, wherein, in order to increase gene expression, one or more nucleic acids encoding a lanosterol C14-demethylase are introduced into the organism.
13. (Withdrawn) The method as claimed in claim 12, wherein the nucleic acids introduced are nucleic acids encoding proteins comprising the amino acid sequence SEQ. ID. NO. 6 or a sequence derived from this sequence by substitution, insertion or deletion of amino acids which has at least 30% identity with the sequence SEQ. ID. NO. 6 at the amino acid level, which proteins have the enzymatic characteristic of a lanosterol C14-demethylase.
14. (Withdrawn) The method as claimed in claim 13, wherein a nucleic acid comprising the sequence SEQ. ID. NO. 5 is introduced.
15. (Currently Amended) The method as claimed in claim 1, wherein, in order to increase the squalene epoxidase activity, the gene expression of a nucleic acid encoding a squalene epoxidase is increased in comparison with the wild type organism.
16. (Previously presented) The method as claimed in claim 15, wherein, in order to increase gene expression, one or more nucleic acids encoding a squalene epoxidase are introduced into the organism.
17. (Currently Amended) The method as claimed in claim 16, wherein the nucleic acids introduced are nucleic acids encoding proteins comprising the amino acid sequence SEQ. ID. NO. 8 ~~or a sequence derived from this sequence by substitution, insertion or deletion of amino acids which has at least 30% identity with the sequence SEQ. ID. NO. 8 at the amino acid level, which proteins have the enzymatic characteristic of a squalene epoxidase.~~
18. (Previously presented) The method as claimed in claim 17, wherein a nucleic acid comprising the sequence SEQ. ID. NO. 7 is introduced.

U.S. Serial No.: 10/549,871
Examiner: Mohammad Y. Meah
GAU: 1652
Page 5 of 14

19. (Withdrawn) The method as claimed in claim 1, wherein, in order to increase the squalene synthetase activity, the gene expression of a nucleic acid encoding a squalene synthetase is increased in comparison with the wild type.
20. (Withdrawn) The method as claimed in claim 19, wherein, in order to increase gene expression, one or more nucleic acids encoding a squalene synthetase are introduced into the organism.
21. (Withdrawn) The method as claimed in claim 20, wherein the nucleic acids introduced are nucleic acids encoding proteins comprising the amino acid sequence SEQ. ID. NO. 10 or a sequence derived from this sequence by substitution, insertion or deletion of amino acids which has at least 30% identity with the sequence SEQ. ID. NO. 10 at the amino acid level, which proteins have the enzymatic characteristic of a squalene synthetase.
22. (Withdrawn) The method as claimed in claim 21, wherein a nucleic acid comprising the sequence SEQ. ID. NO. 9 is introduced.
23. (Previously presented) The method as claimed in claim 1, wherein the organism used is yeast.
24. (Currently Amended) The method as claimed in claim 1, wherein, after the cultivation, the organism is harvested and ergosta-5,7-dienol, mevalonate, farnesyl pyrophosphate, geraniol pyrophosphate, squalene epoxide, 4-dimethylcholesta-8,14,24-trienol, 4,4-dimethylzymosterol, squalene, farnesol, geraniol, lanosterol, zymosterone and zymosterol, campesterol, pregnenolone, 17-OH pregnenolone, progesterone, 17-OH-progesterone, 11-deoxycortisol, hydrocortisone, deoxycorticosterone and/or corticosterone and/or its biosynthetic intermediates and/or metabolites are subsequently isolated from the organism.

U.S. Serial No.: 10/549,871
Examiner: Mohammad Y. Meah
GAU: 1652
Page 6 of 14

25. (Withdrawn) A genetically modified organism, where the genetic modification reduces the $\Delta 22$ -desaturase activity and increases the HMG-CoA reductase activity and increases at least one of the activities selected from the group consisting of lano-sterol C 14-demethylase activity, squalene epoxidase activity and squalene synthetase activity in comparison with the wild type.
26. (Withdrawn) The genetically modified organism as claimed in claim 25, where the genetic modification reduces the $\Delta 22$ -desaturase activity and increases the HMG-CoA reductase activity and increases the lanosterol C 14-demethylase activity in comparison with the wild type.
27. (Withdrawn) The genetically modified organism as claimed in claim 25, wherein the genetically modified organism has an increased content in ergosta-5,7-dienol and/or its biosynthetic intermediates and/or metabolites in comparison with the wild type.
28. (Withdrawn) The genetically modified organism as claimed in claim 25, wherein the organism used is yeast.
29. (Currently Amended) A method for the production of ergosta-5, 7-dienol and/or its biosynthetic intermediates and/or metabolites comprising culturing a genetically modified organism ~~as claimed in claim 25~~ where the genetic modification
reduces the $\Delta 22$ -desaturase activity and
increases the HMG-CoA reductase activity and
increases squalene epoxidase activity in comparison with the wild type.
30. (Withdrawn) A method for the generation of a genetically modified organism in which, starting from a starting organism,

U.S. Serial No.: 10/549,871
Examiner: Mohammad Y. Meah
GAU: 1652
Page 7 of 14

the $\Delta 22$ -desaturase activity is reduced and
the HMG-CoA reductase activity is increased and
at least one of the activities selected from the group consisting of lanosterol C14-
demethylase activity, squalene epoxidase activity and squalene synthetase activity is
increased.

31. (New) The method as claimed in claim 8, wherein the nucleic acids introduced are
nucleic acids encoding proteins having at least 30% identity with the sequence SEQ. ID. NO.
4 at the amino acid level, which proteins have the enzymatic characteristic of an HMG-CoA
reductase

32. (New) The method as claimed in claim 16, wherein the nucleic acids introduced are
nucleic acids encoding proteins having at least 30% identity with the sequence SEQ. ID. NO.
8 at the amino acid level, which proteins have the enzymatic characteristic of a squalene
epoxidase.